**Supplemental Figure Legends**

**Supplementary Figure S1. Atg7 DAT Cre mice lack Atg7 selectively in dopamine neurons.** Atg7 transcript was detected with an RNA probe designed against nucleotides 1518-1860 of the Atg7 gene. Atg7 mRNA was detected in the anterior and central substantia nigra (SN) of the control DAT Cre mouse, but not in the Atg7 DAT Cre mouse (only the anterior plane is shown). The close anatomical correspondence between the anterior coronal SN sections from the control DAT Cre mouse and the Atg7 DAT Cre mouse is shown by the matching locations of the medial lemniscus (ML) and the medial terminal nucleus (MT). Although Atg7 mRNA was not detected in the SN of the Atg7 DAT Cre mouse, it was detected in the dentate gyrus (DG), and red nucleus (RN), indicating selective deletion of the *atg7* gene in the SN by DAT-controlled Cre expression. Nissl staining (blue) showed a comparable number of cells in the Atg7 DAT Cre and the Atg7 DAT Cre mice.

**Supplementary Figure S2. Effects of rapamycin on evoked dopamine release and reuptake. A.** Lack of effect of rapamycin (3 μM) on the $t_{½}$ of the signals in **Figure 2** from paired striata from DAT Cre (mean ± sem; control: 718 ± 29 ms, Rapa: 675 ± 22 ms, n = 7) and Atg7 DAT Cre (753 ± 23 ms, Rapa: 743 ± 32 ms, n = 9) mice ($p>0.05$, 2-way ANOVA). **B.** Representative traces from control and rapamycin-treated wild-type slices. Evoked dopamine release from slices from wild-type striatum incubated in ACSF containing 3 μM rapamycin (n=10) was significantly reduced compared to controls (n=10). As observed in the DAT Cre line (**Figure 2**), rapamycin decreased the peak amplitude of dopamine signals by 40% (mean peak amplitude: 3.7 ± 0.3 μM in controls vs. 2.2 ± 0.3 μM in rapamycin-treated slices).

**Supplementary Figure S3. Effects of rapamycin on protein expression in the acute striatal slice. A**. Representative western blots of proteins from striatal slices obtained from littermate DAT Cre and Atg7 Dat Cre mice following DMSO as vehicle (C) or rapamycin (R: 3 μm, 7 h). Levels of the proteins are displayed in Table 1 as mean ± sem: DAT and TH levels were significantly different between genotypes whereas none of the proteins were significantly altered by rapamycin at 7 h exposure (although there is a transient increase in LC3-II in this preparation observed at 3.5 hours as shown on **Figure 3D**).

**Supplementary Table I.**

| | DAT Cre | | Atg7 DAT Cre | | | | |
|---|---|---|---|---|---|---|---|
| | DMSO | RAPA | DMSO | RAPA | | | |
| | | | | | interaction | genotype | treatment |
| LC3-II | 30 ± 5 | 28 ± 6 | 20 ± 4 | 22 ± 4 | n.s. | n.s. | n.s. |
| DAT | 31± 3 | 34 ± 2 | 15 ± 1 | 20 ± 4 | n.s. | 0.01 | n.s. |
| TH | 26 ± 2 | 29 ± 1 | 20 ± 2 | 25 ± 2 | n.s. | 0.02 | n.s. |
| porin | 26 ± 4 | 23 ± 2 | 24 ± 3 | 26 ± 1 | n.s. | n.s. | n.s. |
| tomm20 | 24 ± 3 | 25 ± 1 | 22 ± 2 | 28 ± 4 | n.s. | n.s. | n.s. |
| PSD95 | 22 ± 4 | 22 ± 4 | 27 ± 3 | 28 ± 3 | n.s. | n.s. | n.s. |

**Table I.** Levels (mean ± sem, n=3) of protein expression measured by western blotting (**Figure 7**) following 7 h exposure of acute striatal slices to vehicle (DMSO) or 3 μM rapamycin (RAPA). In each experiment, striatal slices obtained from the same mouse were exposed to the drugs (n=3 mice per group). Statistics are shown for two factor ANOVA, with genotype (DAT Cre vs. Atg7 DAT Cre) and treatment (DMSO vs. rapamycin) and interaction between the two factors: differences $p > 0.05$ are labeled non-significant (n.s.). Note that LC3-II is increased by rapamycin at 3.5 h, but returns to baseline levels by 7 h (see **Figure 3d**).

**Methods**

*Behavioral assays.* For the *tail hang test*, the mice were suspended in the air by their tails and the clasping of their hind legs was evaluated. Animals displaying standard escape response were given a score of zero whereas animals that maintained legs in clasped or dystonic position were given a score of two. For the *beam walking test* each mouse was placed on the center of a 13-mm-wide square metal beam suspended between two safe platforms (60 cm apart) located 50 cm above a padded surface. The latency to fall was recorded on each of three trials (a maximum fall latency of 60 s was scored for mice which had reached the platform in less than 60 s). For the *open field test* a mouse was placed in the center of the open field arena and allowed to freely move for 30 minutes while being tracked by an automated tracking system (Med Associates Inc., USA). For each 5 min interval several parameters were calculated including total distance traveled, average velocity, ambulatory counts, number of ambulatory episodes and vertical counts. Data were compared using two-way ANOVA.

*Perfusion and sample preparation for electron microscopy*. Mice were injected intraperitoneally with vehicle (DMSO) or rapamycin (2.0mg/kg). Mice were anesthetized with ketamine/xylazine, and a needle connected to a perfusion pump was inserted into the left ventricle. Saline containing heparin was perfused at 4ml/min for 5min to wash out the blood. 2% glutaraldehyde was perfused at 4ml/min for 5min. The fixed brain was then carefully removed from the skull. The striatum was dissected and post-fixed in 2% glutaraldehyde for one hour at room temperature. Sections (40-50 µm) were cut with a vibratome and incubated for 30 min at room temperature in a blocking solution containing 10% normal goat serum in phosphate buffered saline (PBS). The sections were incubated for 48 h at 4° C with a rabbit antibody to TH (diluted 1:2000; Protos Biotech) in PBS containing 10% goat serum. Bound primary antibodies were located with biotinylated secondary antibodies and peroxidase-labeled avidin (Vector ABC elite kit) and peroxidase activity was visualized with 3-3'-diaminobenzidine (DAB). The $H_2O_2$ for the DAB reaction was generate with glucose and glucose oxidase to minimize tissue damage. Sections were finally washed with PBS, treated with 1% $OsO_4$ for 1 h at RT, washed again in PBS and maleate buffer (5x5 min) at RT and stained en bloc with 2% aqueous uranyl acetate on ice for 1 h. Stained sections were dehydrated through a graded series of ethanol solutions, cleared with propylene oxide, and embedded in Epon

812 (Electron Microscopy Sciences). Thin (silver) sections were cut, picked up on copper grids, and examined with a JEOL 1200EX electron microscope.

*Striatal slice preparation.* Mice were sacrificed by cervical dislocation. Striatal slices were cut on a vibratome at a thickness of 300 μm for physiology and 200 μm for electron microscopy. Slices were placed in a holding chamber containing oxygenated ACSF at room temperature and were allowed to recover for at least 1hr before the start of the experiments. Slices were incubated in 3 μM rapamycin or DMSO for at least 6 h before recordings began. For cyclic voltammetry, slices were placed in a recording chamber and superfused with ACSF (in mM): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 0.3 $KH_2PO_4$, and 10 glucose at 36$^oC$.

*5OHDA incubation.* Male mice (3-6 months of age) were euthanized and 200 μm slices prepared as above. Hemislices were transferred to a chamber containing oxygenated ACSF with either DMSO or rapamycin (3 μM) in DMSO for 6.5h. This ACSF contained 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM MgCl, 1.25 mM $NaH_2PO_4$ and 10mM glucose. Slices were transferred to the following solution with 500 μM 5OHDA (Sigma) for 0.5 h: 10 mM NaCl, 2.5 mM KCl, 25 mM HEPES, 1mM $NaH_2PO$, 2 mM $CaCl_2$, 10 mM glucose, 0.2 mg/ml ascorbic acid, 0.16 mg/ml pargyline HCl, pH of 7.4. Slices were then rinsed three times in the same ACSF for 5 min each. The slices were then fixed in ice cold 3% sodium permanganate in the same ACSF for 40 min on ice. Slices were then washed three times for 5 min in the same ACSF at room temperature and then three times for 5 min in 0.9% NaCl. Samples were stained en bloc staining in 4% uranyl acetate on ice for 1 h. Specimens were dehydrated in a graduated series of ethanols, cleared in propylene oxide, and embedded in Spurr medium (Ted Pella).

*Slice preparation for electron microscopy.* Additional slices (with the exception of those processed for 5OHDA as above), prepared for electron microscopy (200 μm thickness for electron microscopy) were placed for 7h in a holding chamber containing oxygenated ACSF containing either DMSO or rapamycin (3 μM). They were fixed in 2.5% glutaraldehyde in 0.1M Sorensen's buffer (pH 7.2) overnight and then washed with 0.1M Sorensen's buffer 3 times for 5 min. Slices were incubated in 1% OsO4 diluted in Sorensen's buffer for 1 h. Slices were then washed in buffer 3 times for 2 min and one time in water. The slices were placed in freshly made 1% tannic acid in water for 15min,

followed by another wash in water. En bloc staining was performed with 1% uranyl acetate in water for 1 h. Dehydration was performed in 50% and 70% ethyl alcohol for 10min and then in 95% and 100% three times for 10 min. Following dehydration, slices were incubated in 2 parts 100% alcohol to 1 part embedding medium (Ladd LX-112 & EMS 112) for at least 1 h. Then they were incubated in 1:1 100% alcohol to embedding medium overnight at 60 °C. Dishes were then drained and fresh embedding medium was added for at least 2 h.

*Protein collection.* Male mice (3-6 months of age) were sacrificed as above and 200 µm corticostriatal coronal slices prepared) in 4°C oxygenated solution containing: 10mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 1.25 mM NaH2PO4xH20, 180 mM sucrose, and 10 mM glucose. Hemislices were then transferred to a chamber containing oxygenated ACSF with either DMSO or rapamycin (3 µM) in DMSO for 7 or 3.5h. ACSF contained 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 M $MgCl_2$, 1.25 mM $NaH_2PO_4$, and 10mM glucose. After incubations the cortex was separated from the striatum and discarded. Sets of 2 hemislices were collected into individual tubes and snap-frozen on dry ice. Tubes were stored at -80°C for 1-3 days.

*Western blot.* Tubes were removed from -80°C and the striatal tissue sonicated in 100 µL of 1% SDS. Contents were transferred into eppendorf tubes and boiled at 100°C for 5 min. Protein concentration was determined by the BCA method (Pierce). A total of 25ug were loaded in each well of a 15% bisacrylamide gel and run at 180 mV for 1 h or until the protein ladder markers (Fisher) were well separated. Protein was transferred to a PVDF membrane at 40 mV for 3h or at 10 mV overnight. Membranes were then rinsed and washed once in 1X TBS/0.05% Tween-20, blocked in 5% milk and incubated with the following primary antibodies: 1:60000 anti-mouse beta-actin (Sigma), 1:500 anti-rat DAT (Millipore), 1:1000 anti-rabbit LC3 (Novus), 1:2000 anti-guinea pig p62 (American Research Products), 1:1000 anti-mouse porin (Mitosciences), 1:1000 anti-PSD95 (Abcam), 1:2000 anti-mouse TH (Millipore), 1:5000 anti-mouse tim23 (BD Biosciences), and 1:333 anti-mouse tomm20 (Abcam). Corresponding HRP-conjugated goat secondary antibodies were used at a 1:10000 concentration; anti-mouse and anti-rabbit (Thermoscientific), anti-guinea pig and anti-rat (Novus). Protein was detected by incubating membranes for 5 min with HRP substrate (Millipore), and Image J was used to determine the intensity of bands using actin to normalize the signal. For comparison

between DAT Cre and Atg7 DAT Cre lines, a pair of littermates was compared per experiment using 2-4 slices per animal, and the levels from each slice combined and divided by the number of slices. Each experiment was conducted at least three times, and data in Table I and Figure present the mean and s.e.m. as percent of total signal intensity obtained per experiment.

*Electrochemical recordings.* Electrical stimulation and recording protocols were adapted from Schmitz et al., 2001. Carbon fiber electrodes (5 µm diameter) were placed in the dorsal striatum, approximately 50 µm into the slice. For cyclic voltammetry, a triangular voltage step (-400 mV to +1000 mV at 300 V/s vs Ag/AgCl) was applied to the electrode every 100ms. Currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), with a low-pass Bessel Filter setting at 5 kHz, digitized at 25 kHz (ITC-18 board, Instrutech Corporation, Great Neck, New York). We used IGOR for data acquisition (WaveMetrics, Lake Oswego, OR) with a locally written program (Dr. E. Mosharov, Columbia University) available at http://sulzerlab.org/download.html.

Striatal slices were stimulated every 2 min with either a single pulse of electrical stimulation or paired stimuli via an Iso-Flex stimulus isolator triggered by a Master-8 pulse generator (A.M.P.I., Jerusalem, Israel) using a bipolar stimulating electrode placed approximately 100µm from the recording electrode. To assess single pulse (1p) evoked dopamine release, slices were stimulated in the dorsal striatum with a single pulse of electrical stimulation (0.4mA, 1ms duration). Three or four individual recordings were taken from each of 3 sites on each slice. To calculate the 1p evoked release from each slice all 9-12 recordings were averaged together. To obtain the paired pulse ratio (PPR), slices were stimulated with 2 pulses of electrical stimulation separated by interstimulus intervals of 1, 2, 5, 10, 20, 30, and 60s. To calculate the PPR, the peak from the second stimulation was normalized to the first. Background-subtracted cyclic voltammograms served to identify the released substance. The dopamine oxidation current was converted to concentration based on a calibration of 5 µM dopamine in ACSF after each slice.

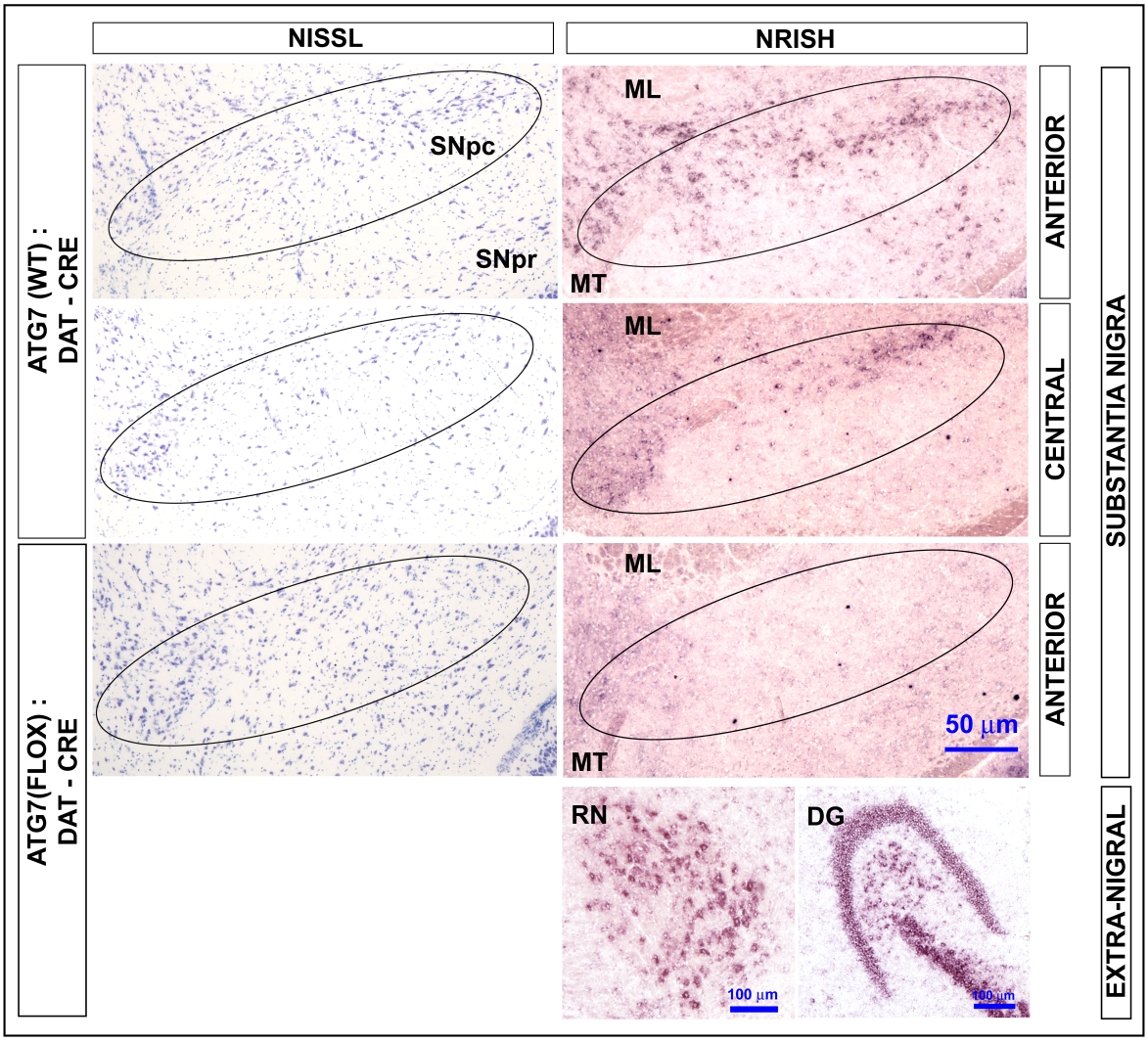
*Non-radioactive in situ hybridization*. Tissue was mounted in OCT and frozen sections (14 µm) were prepared with a cryostat and mounted on SuperfrostPlus slides (Fisher 12-550-15). They were kept at -80°C until used. Tissue was fixed in freshly prepared 4% paraformaldehyde, 0.1 M PBS for 10 min at room temperature. Samples were then

washed 3 times for 5 min in PBS. Sections were then treated with a pre-hybridization solution as previously described (Burke et al., 1994) for 2 h at room temperature. Sections were then covered with hybridization solution and incubated overnight at 68ºC. Hybridization solution contained either anti-sense or sense ATG7 probe (Cheng et al, 2011) labeled with digoxigenin-UTP, prepared as per the manufacturer's instructions (Roche Diagnostics). The size and integrity of labeled probe were confirmed by gel electrophoresis. After washes in 0.2xSSC at 68ºC, sections were incubated with an anti-digoxigenin antibody (Roche) at 1:5000 overnight at 4ºC. After additional washes, sections were incubated with a developing solution containing BCIP/NBT (Promega) overnight at room temperature in the dark. Sections were washed and coverslipped with DAKO aqueous mounting medium.

**References:**

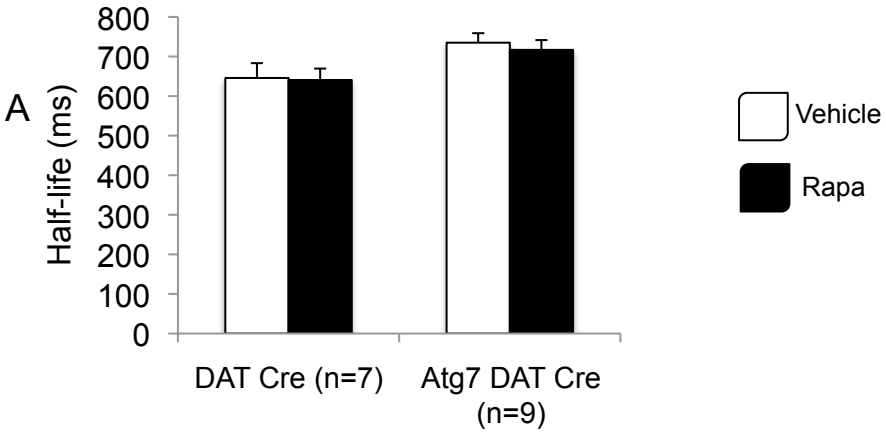
Burke RE, Franklin SO, Inturrisi CE. Acute and persistent suppression of preproenkephalin mRNA expression in the striatum following developmental hypoxic-ischemic injury. J Neurochemistry 1994;62:1878-1886.

Cheng H-C, Kim SR, Oo TF, Kareva T, Yarygina O, Rzhetskaya M, Wang C, During MJ, Talloczy Z, Tanaka K, Komatsu M, Kobayashi K, Okano H, Kholodilov N, Burke RE. Akt suppresses retrograde degeneration of axons by inhibition of macroautophagy. Journal of Neuroscience, 2011, 31:2125-2135. (PMCID: PMC3075005).




Supplementary Figure 1

A

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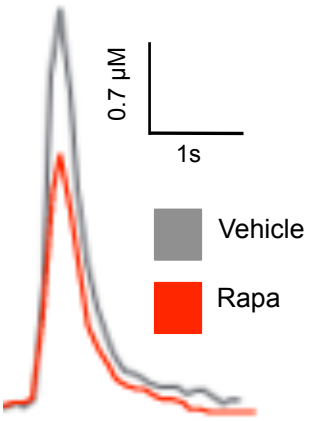
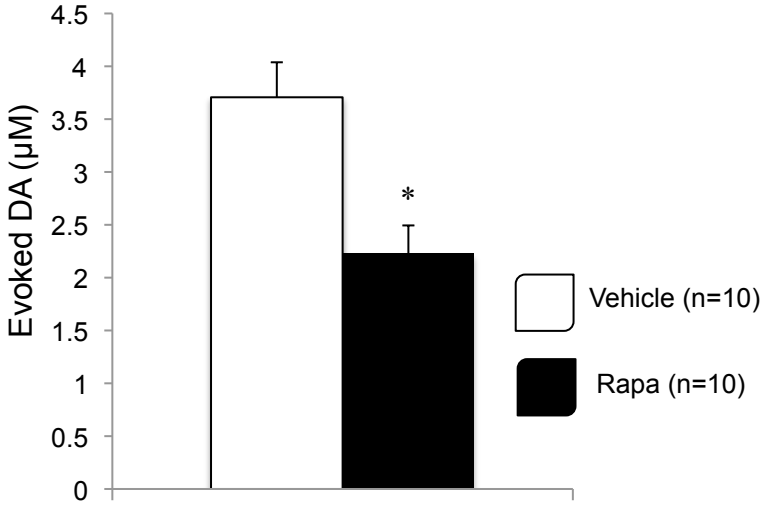
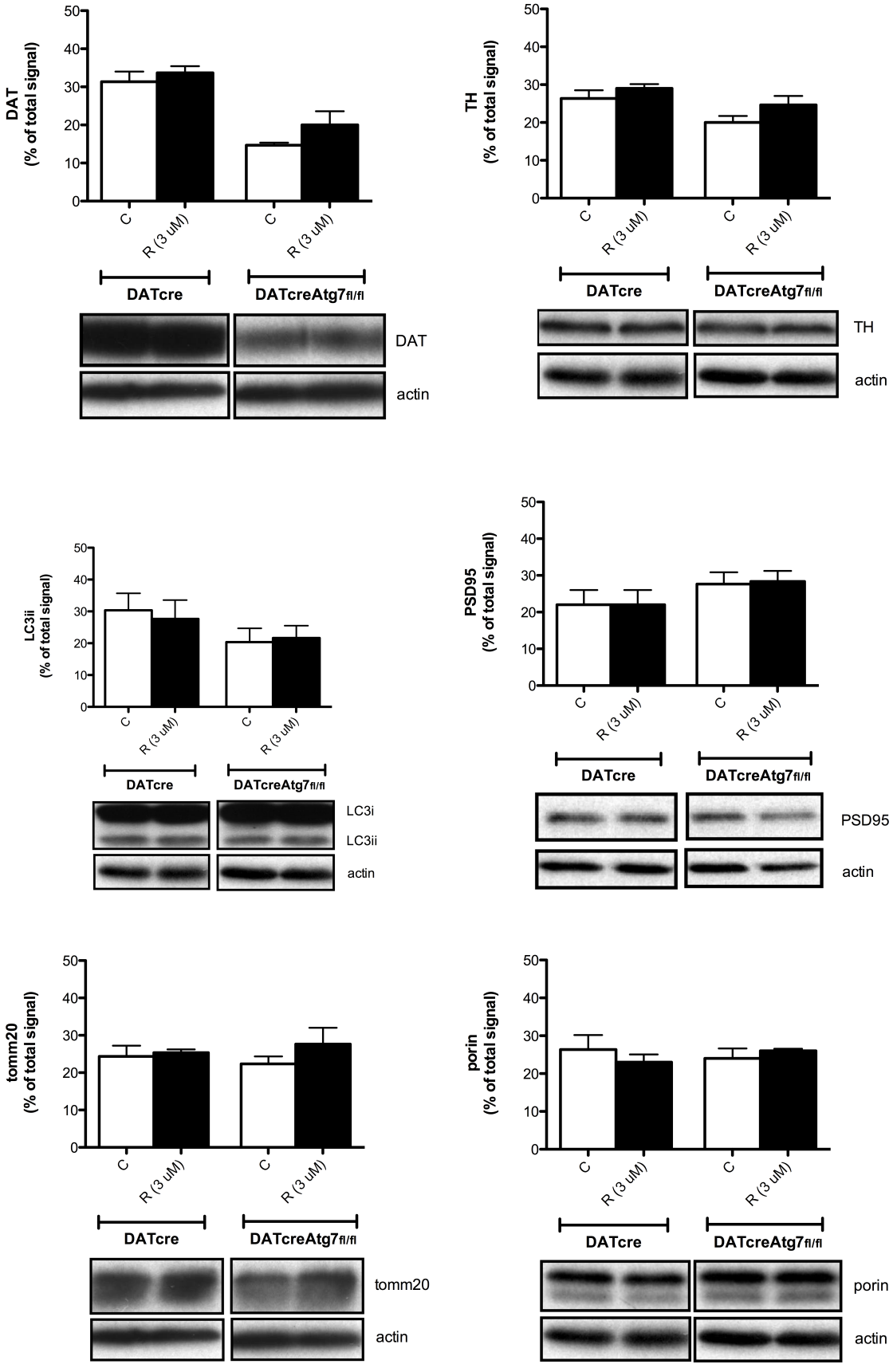



Supplementary Figure S2




Supplementary Figure S3